Assuming the rate of progress during the first year is as estimated above, our second year's work would be concerned with the study of the "fragments." At this point it may prove necessary to work with C-14 labeled nicotine in order to pinpoint the fate of the individual carbons in the nicotine molecule. Since this could involve the synthesis of specifically labeled nicotine, the proposed materials budget for the second year has been increased. Having somewhat underestimated the time factor in previous grant requests, I hesitate to state where we should be at the end of the two years proposed. I feel however that we should be fairly near to the overall completion of the project.

6. Budget Plan:

	1st yr.	2nd yr.
Salaries	8,400.	3,600.
Expendable Supplies	1,000.	2,000.
Permanent Equipment	-	-
Overhead (8%)	780.	476.
Other (Travel)	350.	350.
Total	$10,530.	$6,426.

7. Anticipated Duration of Work:

Two years to complete the work outlined. Perhaps one additional year to complete the project as originally conceived.

8. Facilities and Staff Available:

Complete facilities available for work proposed.

Staff: Dr. S. C. Rittenberg, Professor of Bacteriology, Director
Dr. S. H. Richardson, Research Associate, full time at $6,000 per year. Dr. Richardson has worked on this project for two years and has just completed all requirements for his Ph.D. degree.
Mr. R. Gherna, Research Assistant, approximately half time at $2,400 per year. Mr. Gherna has worked for two summers on this project as an undergraduate assistant. He will start work toward his M.A. degree in Sept. 1960.

9. Additional Requirements: None

10. Additional Information:

See previous grant applications and semiannual and annual reports.

Signature Sydney C. Rittenberg
Director of Project

Business Officer of Institution

Committee
Jacobson, Chairman
Kotin
Little

#209R2
Activated 10/1/58
Renewed 10/1/59
Cf. #86A
Activated 10/1/56
Renewed 10/1/57

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street New York 17, N.Y.

Application For Research Grant

Date: May 13, 1960

1. Name of Investigator: Sydney C. Rittenberg, Ph.D.

2. Title: Professor of Bacteriology

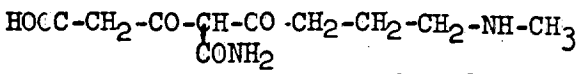
3. Institution & Address: University of Southern California
Los Angeles 7, California

4. Project or Subject: The Bacterial Oxidation of Nicotine.

5. Detailed Plan of Procedure:

Our studies on the bacterial oxidation of nicotine have been supported by a series of grants from the TIRC since Oct. 1, 1956. The detailed plan of procedure and results obtained have been presented to the Committee in previous grant applications, in semiannual and annual reports, and in a series of articles in the Journal of Biological Chemistry. In general, we expect to continue our investigations along the lines previously followed. Specifically, during the remaining months of the current grant and in the following year, we expect (1) to complete the identification of the blue pigment, (2) to isolate and identify 4th and 5th product, and (3) to survey by the simultaneous adaptation technique a group of 10-15 nicotine-oxidizing bacteria to determine if the metabolic pathway of the bacterium we are studying is common to most or all nicotine-oxidizing bacteria. The background for the above goals was given in the last semi-annual report and will not be repeated here.

From the evidence already available it seems almost certain that both the pyridine and pyrrolidine rings have been opened by the end of the 5th step. A reasonable guess is that a molecule like the following exists at this stage:



The position chosen for the amide and carbonyl group on what was the pyridine ring may not be correct. However, it is clear that labile sites must exist and further oxidative steps should result in rapid fragmentation of the chain, forming small molecules like methylamine, malonate, succinate, acetate, butyrate and propionate. Since the metabolic pathways of such compounds are reasonably well known, our original goal would be achieved as soon as the fragments of further oxidation are identified as to nature and carbons of origin.

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